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Nitrous Oxide Emissions in a Wheat/Corn Succession Combining Dairy Slurry and Urea as Nitrogen Sources

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ABSTRACT: The impact on nitrous oxide (N_2O) emissions caused by combining dairy slurry (DS) and urea to supply crops with nitrogen (N) is still not well characterized. The main objective of this study was to compare the differences between N_2O emissions in the cases of exclusive use of urea and the combined use of DS and urea as N sources to no-tillage wheat and corn. We also compared N_2O emissions between two DS application methods (surface-broadcast vs. injection), as well as the addition of dicyandiamide (DCD) to DS. The experiment was conducted under no-tillage and six treatments were applied at sowing as follows: no fertilization (control), surface-broadcast urea (urea-N), surface-broadcast DS (DSs), surface-broadcast DS with DCD (DSs + DCD), shallow-injected DS (DSi), and shallow-injected DS with DCD (DSi + DCD). Urea was applied at side-dressing in all treatments with DS and urea-N. The N_2O emissions were evaluated from the application of the treatments to wheat until 22 days after corn harvest, resulting in a total of 364 days. The partial supply of the N demand of wheat and corn, by DS application at sowing, and the posterior complement of N demand by side-dressing urea increased the annual N_2O emissions only when DS was shallow-injected without DCD (DSi). Although the amount of N_2O-N emitted from the DSi treatment was 1.04 kg ha^{-1} (91.2 %) higher than from DSs in corn, the annual N_2O-N emissions did not differ between the two methods of DS application to the soil. The emission factors of N_2O-N were low, ranging from 0.49 % (DSi + DCD) to 1.27 % (DSi). The results of this study suggest that the combined use of DS with urea in N fertilization of no-tillage wheat and corn (DS applied at sowing and urea at side-dressing) caused no changes in the annual N_2O-N emissions compared to crops fertilized exclusively with urea (1/3 at sowing + 2/3 at side-dressing). However, DCD should be added if DS is shallow-injected.

Keywords: dicyandiamide, DCD, slurry injection in soil, no-tillage.

INTRODUCTION

Brazil is the sixth largest producer of cow milk in the world, with a herd of approximately 40 million bovine animals (Anualpec, 2014), which generates large amounts of slurry in the surroundings of milking facilities. This slurry consists mainly of the mixture of feces and urine, in addition to the water from the cleaning of the premises, and it is not only a promising source of nutrients to crops (Barcellos, 1991; Jokela et al., 2014), but also a potential environmental polluter (Burkhart and James, 1999; Dalal et al., 2003).

One of the properties of dairy slurry (DS) is its low total N content. When analyzing 47 DS samples collected in the state of Rio Grande do Sul, 41 of which came from dairy cows, Barcellos (1991) found a wide variation in the total N content (0.15 to 3.67 kg m^{-3} N) and an average value of only 1.42 kg m^{-3} N. With the current expansion of agroecological milk production in southern Brazil (Balem and Machado, 2014), the levels of N in DS should be even lower, because the feeding of the cows in this system is based on the use of perennial pastures in improved native grassland plus a complementary diet in the form of dry fodder during milking.

The average content of 1.42 kg m^{-3} N in DS found by Barcellos (1991) is an example of the difficulty in meeting the high N demands of some species (e.g., corn) exclusively with DS. Based on the hypothesis that it would be recommended to apply a dose of 120 kg ha^{-1} N to corn and considering a 50 % efficiency index for the N of DS (CQFS-RS/SC, 2004), this would imply applying 240 kg ha^{-1} of total N, with 169 m^3 ha^{-1} of slurry, which is technically difficult and environmentally risky. Therefore, the use of excessive DS rates to cereals could be avoided by only partially supplying the N crop demand with DS applied at sowing, and the remaining N by side-dressing urea-N. However, no research results were found in Brazil involving this fertilization strategy in cereals.

In no-tillage (NT) systems, used on approximately 80 % of the agricultural area of southern Brazil (Amado et al., 2006), animal slurry must be applied to the soil surface. Prior to application, the produced slurry is stored mainly in anaerobic tanks, where the mineralized N remains in ammoniacal (NH$_3$ + NH$_4^+$) form and can reach 40 to 50 % of the total N of the slurry (Thompson and Meisinger, 2002). It is known that slurry application to the soil surface without incorporation potentiates N losses by ammonia (NH$_3$) volatilization (Carozzi et al., 2013; Aita et al., 2014), reducing the potential of slurry in supplying N to crops (Gonzatto et al., 2016), and contributing indirectly to N$_2$O emission (IPCC, 2006). When DS was applied to the soil surface under different soil and climate conditions, the proportion of ammoniacal N lost by NH$_3$ volatilization from DS was quite variable, ranging from 22 % in winter (Martínez-Lagos et al., 2013) to 82 % in summer (Salazar et al., 2014). Although the mechanical incorporation of DS into the soil shortly after application reduces the action of temperature and wind, protecting ammoniacal N from volatilization (Carozzi et al., 2013), this practice is incompatible with NT (Rochette et al., 2009; Maguire et al., 2011). Thus, an alternative is the injection of dairy slurry into the soil in NT systems, with a reduction in NH$_3$ volatilization, which was 70 % after pig slurry injection in corn (Aita et al., 2014) and 76 % after DS injection into native pasture (Häni et al., 2016).

The positive effect of injecting animal slurry into the soil to reduce NH$_3$ volatilization can be offset by the increase in N$_2$O emissions, as found in studies with DS (Velthof and Mosquera, 2011) and pig slurry (Aita et al., 2014) under no-tillage. The reason is that injection increases the water soil content and concentrations of soluble carbon (C) and available N in the injection slots, favoring N$_2$O production from nitrification and especially denitrification (Klein et al., 1996; Webb et al., 2010). Although after the DS application to soil N$_2$O emission in quantitative terms is generally lower than 2 % (Louro et al., 2013; Bell et al., 2015; van der Weerden et al., 2016), it is relevant from an environmental point of view. This is because aside from being a potent greenhouse gas, with a global warming power nearly 310-fold that of CO$_2$ (Cui et al., 2011), N$_2$O is also associated with the depletion of the ozone layer (Stocker et al., 2013). Thus, we must seek strategies that can mitigate N$_2$O production and emission in agricultural applications of animal slurry, especially under no-tillage conditions.

One of the strategies that have been evaluated is the addition of nitrification inhibitors to slurry, especially dicyandiamide (DCD). Some studies with pig slurry in Brazil (Aita et al., 2014) and other countries (Vallejo et al., 2005; Dell et al., 2011) have shown a reduction in N_2O emissions when slurry is injected into the soil along with DCD. By inhibiting the action of ammonia-monooxygenase (AMO) during the first nitrification step, DCD reduces N_2O production both through the nitrifier denitrification process when the soil availability of O_2 and C is low (Wrage et al., 2001), and denitrification under anaerobic conditions (Morley and Baggs, 2010). However, for the conditions in Brazil, no research results about the effect of DCD addition to DS on soil N_2O emissions were found.

This study was based on the hypothesis that the strategy of partially supplying the N demand of wheat and corn by DS at crop sowing and complementing N by side-dressing urea results in N_2O emissions equivalent to the supply of N exclusively with urea, provided that DCD is added to DS. Thus, the objective was to compare the annual N_2O emissions arising from the use of urea as an exclusive source of N to wheat and corn with the N fertilization strategy combining DS application at sowing and side-dressed urea, with two DS application methods (injection × surface broadcast), with and without addition of DCD to DS.

MATERIALS AND METHODS

The study was done on a field experiment conducted from May 2015 to May 2016 in the experimental area of the Soils Department of the *Universidade Federal de Santa Maria* (UFSM), in the municipality of Santa Maria, RS (29° 42' 50.92" S, 53° 42' 25.55" W, 80 m a.s.l.). The climate is humid subtropical (Cfa), with an average annual temperature of 19.3 °C and average annual rainfall of 1,561 mm. The soil is classified as *Argissolo Vermelho Distrófico arênico* - Hapludalf (Santos et al., 2013; Soil Survey Staff, 1999). At the installation of the experiment, the soil had the following properties (0.00-0.10 m layer): 147 g kg^{-1} clay; 687 g kg^{-1} sand; 5.3 pH(H$_2$O); 13.2 mg dm^{-3} P, and 72 mg dm^{-3} K (both extracted by Mehlich-1); base saturation of 41.9 %; 6.5 cmol$_c$ dm^{-3} CEC$_{pH7}$, and soil density of 1.64 Mg m^{-3}. The values of total C (7.6 g kg^{-1}) and total N (0.7 g kg^{-1}) were determined by dry combustion in an elemental analyzer (FlashEA 1112, Thermo Electron Corporation, Milan, Italy).

The area was left fallow for one year prior to the start of the experiment, and in the two years before the fallow period, cultivated with no-tillage soybean [*Glicine max* (L.) Merr.] in summer and ryegrass (*Lolium multiflorum* L.) in winter. After mechanical mowing and chemical desiccation of the area with glyphosate, the experiment was initiated in June 2015 with treatments applied to wheat (*Triticum aestivum* L.) and ended in May 2016 after the corn (*Zea mays* L.) harvest. A randomized block design with four replications and plots of 33.0 m^2 (6.0 × 5.5 m) was used.

Nitrous oxide emissions were evaluated for two DS application methods to the soil, as sowing fertilization in a no-tillage system, with and without addition of the nitrification inhibitor dicyandiamide (DCD) to DS. All strategies of DS application at sowing were combined with N (urea) application as side-dressing, because urea is the N source most commonly used by farmers. The two DS application methods to the soil at sowing consisted of: surface-broadcast DS (DSs) and shallow-injected DS (DSi). These strategies of N fertilization were compared to the traditional, i.e., urea application at sowing and side-dressing, with no other N sources.

Fertilization treatments applied at sowing consisted of: T1 = no fertilization (Control); T2 = surface-broadcast urea-N (Urea-N); T3 = surface-broadcast DS (DSs); T4 = surface-broadcast DS with DCD (DSs + DCD); T5 = shallow-injected DS (DSi); and T6 = shallow-injected DS with DCD (DSi + DCD). All DS treatments were accompanied by posterior urea side-dressing, to complement the crop N demand. The treatments were evaluated in one growing season, applied to wheat (winter 2015) and reapplied to the same plots with corn (summer 2016).

Amounts of fertilizers applied to wheat and corn (Table 1) were calculated based on the recommendation for these crops (CQFS-RS/SC, 2004) for an expected grain yield of 3 and 6 Mg ha^{-1} for wheat and corn, respectively. The amount of N in treatment T2 (urea-N) was divided into two parts: 1/3 at sowing and 2/3 at side-dressing, both applied on the soil surface. In this treatment, aside from the urea applied at sowing, P and K were also applied (both on the soil surface) as triple superphosphate and potassium chloride, respectively.

The DS rates applied to wheat and corn at sowing were calculated based on the total N content of DS, considering that 50 % of the total N would be available during the cycle of each crop (CQFS-RS/SC, 2004). In all fertilization treatments, urea was applied by hand to the soil surface at side-dressing.

Dairy slurry was produced by lactating cows that spend most of the time on native grassland paddocks in a rotational grazing system. The cows were brought in for milking twice a day and fed with corn silage and dry feed in troughs. The slurry consisting of feces, urine, food residues, and water from the cleaning of the premises. The slurry was stored in a concrete storage tank on a dairy farm and transported to the experimental area one week before the treatments were applied, where the DS was stored in 1000 L water tanks. The total solids content in DS was determined by weighing an aliquot of 80 mL after drying in an oven with air circulation at 70 °C for 48 h. The total N and total ammoniacal N (TAN = NH_3 + NH_4^+) contents were determined in slurry without previous drying, by wet digestion and MgO distillation, respectively (Tedesco et al., 1995). The contents of P and K in DS were determined by colorimetry and photometry, respectively (Murphy and Riley, 1962). The total C content in DS (dried at 70 °C and ground) was determined by dry combustion in an elemental analyzer (FlashEA 1112, Thermo Electron Corporation, Milan, Italy), while the pH was measured directly in an aliquot of 80 mL of slurry. The main properties of DS applied to wheat and corn are presented in table 1.

Wheat (cv. Tbio Sinuelo) treatments were applied on June 16, 2015. Sowing (160 kg ha^{-1} of seeds) was done on June 22, 2015 and grain harvest on November 2, 2015. The application of side-dressed urea in treatments with DS and urea-N was carried out 26 days after the sowing of the crop. On the 24th day after the wheat harvest, wheat residues were added to the bases used to evaluate N_2O in the post-harvest period and during the subsequent corn. The amount of dry matter (DM) of wheat residues added was equivalent to its production in each treatment.

Corn (Híbrido Agroeste AS 1551 PRO2) crop was sown on January 7, 2016, three days after the application of the treatments, at a spacing of 0.70 m between rows and a quantity of seeds to ensure a final plant density of approximately 60 thousand plants ha^{-1}. The addition of side-dressed urea in treatments with DS and the urea-N treatment were applied to the soil surface 30 days after sowing. Corn was harvested for grain yield evaluation on May 23, 2016. In both crops, fertilization with P and K was applied by hand to the soil surface at sowing.

The treatments with DS on the soil surface were applied by hand with 10 L ewers. On the other hand, DS injection treatments were applied with a mechanical slurry injector (Model DAOL-i 4000 Tandem, MEPEL, Estação-RS, Brazil). The equipment consists of a 4,000 L metallic tank and injection begins after the activation of a hydraulic piston by which a set of eight guillotine-type injection rods (cutting discs and grooving rod) located on the back of the machine are driven into the soil. The 2.0 cm thick grooving rods have a replaceable tip and are spaced 0.35 m apart, and the depth of the injection of DS into the soil varied from 0.08-0.11 m. The nitrification inhibitor used was DCD, which was mixed with DS in a 1000 L water tank at a rate of 10 kg ha^{-1}, no earlier than one hour prior to DS application to the soil.

Nitrous oxide emissions were evaluated using static chambers, as described by Aita et al. (2014), from treatment application to wheat until 22 days after the corn harvest, i.e.,

Table 1. Main properties of dairy slurry (DS) and amounts of nitrogen (N), phosphorus (P) and potassium (K) applied in the treatments with DS and Urea-N treatment in wheat and corn

Variables	Wheat 2015	Corn 2016
Properties of the DS[1]		
Dry matter (g kg^{-1})	35.5	13.8
Total C (g kg^{-1})	357.8	306.7
Total N (g kg^{-1})	1.14	0.71
TAN[2] (g kg^{-1})	0.27	0.38
pH	6.9	7.2
Treatments with DS		
Dose (m^3 ha^{-1})	80	83
Total N (kg ha^{-1})	91.2 (45.6)[3]	58.9 (29.5)
TAN[2] (kg ha^{-1})	21.6	31.5
Total P (kg ha^{-1})	32.0	27.4
K (kg ha^{-1})	79.2	91.3
Urea-N[4] (kg ha^{-1})	64.4	90.8
Urea-N treatment		
Urea-N (kg ha^{-1})	110	120
K-K$_2$O (kg ha^{-1})	33.2	41.5
P-P$_2$O$_5$ (kg ha^{-1})	32.7	50.2

[1] Data expressed as wet basis. [2] TAN = total ammoniacal N ($NH_3 + NH_4^+$). [3] Values in parentheses refer to the amount of N of the DS that will be made available during the growing season of each crop, as recommended by CQFS-RS/SC (2004). [4] In the treatments with DS, urea-N was applied only at side-dressing. In the urea-N treatment, urea was split (1/3 at crop sowing and 2/3 at side-dressing). Dry matter, total N, TAN, and pH were determined according Tedesco et al. (1995). Total P and K were determined by colorimetry and photometry, respectively (Murphy and Riley, 1962). Total C was determined by dry combustion in an elemental analyzer.

a total of 364 evaluation days. Nitrous oxide was evaluated three to four times a week during the first month after the application of the treatments, and then spaced out to once a week. During the experiment, we carried out 35 evaluations in wheat, 30 in corn, and nine in the post-harvest periods. In each N_2O evaluation, four soil subsamples per plot (0.00-0.10 m layer) were collected with a stainless steel auger (diameter 3 cm). The subsamples were mixed to form a single composite sample, of which gravimetric moisture and mineral N content (NH_4^+ and NO_3^-) were determined. Gravimetric soil moisture was assessed by drying samples in an oven at 105 °C for 24 h (Tedesco et al., 1995). Based on the values of moisture and volumetric density, we calculated the water-filled pore space (WFPS) assuming a particle density of 2.65 Mg m^{-3}. Extraction of the mineral N from the soil was done by mechanically agitating a suspension of 20 g of soil and 80 mL of KCl 1 mol L^{-1} at 120 rpm for 30 min. After 30 min decantation, the solution supernatant was filtered (Whatman No. 42 filter paper) and frozen until analysis in a flow analyzer (San^{++} Automated Wet Chemistry Analyzer, Skalar, Breda, Netherlands).

On each evaluation date, N_2O emissions from the soil surface were measured only once a day, from 9:00 to 11:00 a.m. The average daily N_2O fluxes were calculated as described by Rochette and Bertrand (2008) and the cumulative N_2O-N losses were obtained by linear interpolation of the emissions between the sampling dates. The net N_2O-N emission from treatments with application of N sources was calculated at the end of the study by subtracting the amount of N_2O-N emitted in the control treatment without N application. The emission factor (EF) of N_2O-N for the treatments with application of both N sources (DS and urea) was calculated by dividing the annual net emission of N_2O-N of these treatments by the amount of total N applied with each N source and multiplying the result by 100.

The results were tested for normality by the Kolmogorov-Smirnov method and subjected to analysis of variance. When the effects were significant, the means were compared by the Least Square Difference (LSD) test at 5 %.

RESULTS AND DISCUSSION

N₂O fluxes

Fluxes of N_2O increased with the application of DS at wheat and corn sowing and the subsequent application of side-dressed urea in all treatments compared to the control treatment (Figure 1a). However, this increase was limited to the first days after application of the two N sources, when there was also an increase in the availability of soil NH_4^+ (Figure 1b) and NO_3^- (Figure 1c), demonstrating the importance of soil mineral N in controlling N_2O production through microbial nitrification and denitrification processes (Subbarao et al., 2006). The decrease in N_2O fluxes after the application of N in soil, which rapidly returned to the values found before the application of the treatments, was also observed in other studies with cattle slurry (Bell et al., 2015) and pig slurry (Aita et al., 2014). The results found in these studies were attributed to a rapid decrease in the availability of C and N for to the microorganisms responsible for N_2O production.

During the period in which N_2O fluxes increased due to the application of N sources, there were few peaks in emission, generally associated to the occurrence of rainfall, which increased the WFPS values in the 0.00-0.10 m layer (Figure 1d). In most evaluations, the WFPS exceeded 60 %, which is considered the lower limit for N_2O production by denitrification (Linn and Doran, 1984).

The higher amount of urea-N applied by side-dressing to corn than wheat (Table 1) resulted in higher amounts of mineral N in soil, especially as NO_3^- (Figure 1c). Nevertheless, the impact of the use of this source of N on N_2O fluxes was lower in corn. It is likely that the low occurrence of rainfall in the first days after the application of urea (Figure 1a) maintained an adequate level of O_2 in soil, inhibiting N_2O production. Low N_2O emissions in dry periods after application of cattle slurry or ammonium nitrate (NH_4NO_3) from natural grassland were also observed by Louro et al. (2013). Even after the reoccurrence of rainfall (Figure 1a), N_2O fluxes in corn remained very low, probably due to the reduction in the availability of substrates (NH_4^+, NO_3^-, and C) for N_2O production. This is most likely the reason for the low N_2O fluxes found in the periods after the harvest of wheat and corn. In one of the few studies in which N_2O emissions after harvest were evaluated, the observed values were low (<5 g ha^{-1} d^{-1} N) and there were no differences between the sources of N applied to corn and the control treatment (without N application) (Halvorson and Del Grosso, 2012).

Cumulative N₂O emissions

The cumulative annual N_2O emissions differed between treatments, increasing from 2.26 kg ha^{-1} N_2O-N in the control treatment to 6.14 kg ha^{-1} N_2O-N in the DSi treatment. All treatments had higher annual N_2O emission than the control treatment, except DSi + DCD (Figure 2a). With regard to cumulative annual net emissions of N_2O, deducting the emissions of the control treatment (Figure 2b), we found higher emission for DSi than the other treatments, except for DSs. Although DSi exceeded DSs by 1.43 kg ha^{-1} N_2O-N (58 %), this difference was not significant. This can be attributed to the high spatial variability typically observed in N_2O production in soil (van Groenigen et al., 2004). The difference in cumulative N_2O emission between DSi and DSs became more marked after the application of DS to corn (Figure 2). This is probably due to the increase in the availability of C in soil derived from DS, both due to the heterotrophic microbial population in general and to the denitrifying bacteria, combined with the high levels of mineral N, occurring as NO_3^- in the soil (Figure 1c).
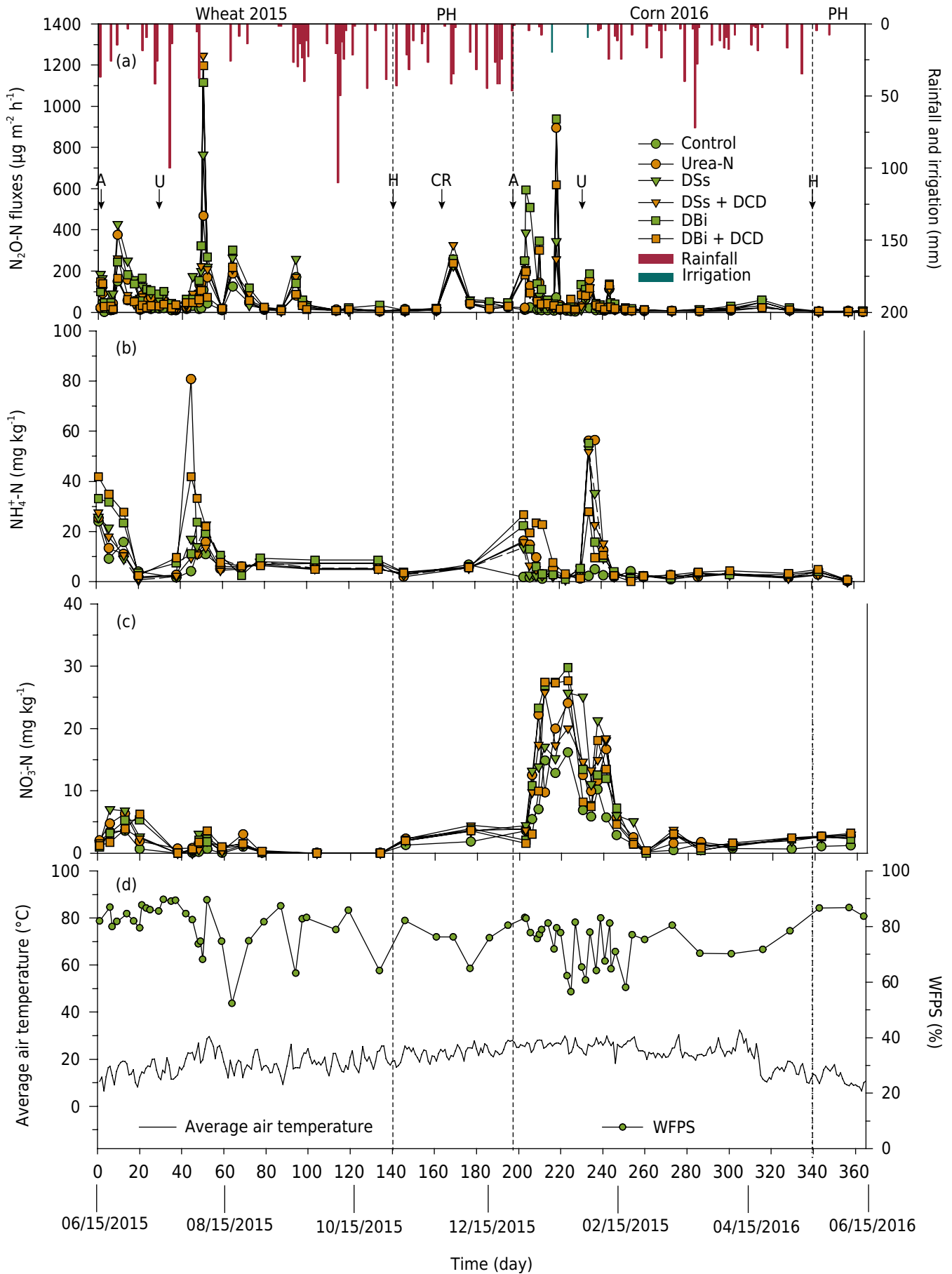
Figure 1. Nitrous oxide emission (a), contents of NH_4^+ (b) and NO_3^- (c), and water-filled pore space (WFPS) (d) in the 0.00-0.10 m soil layer after the application of treatments in wheat and in corn and during the post-harvest (PH) period. Urea-N = surface-broadcast urea-N; DSs = surface-broadcast DS; DSi = shallow-injected DS; DCD = dicyandiamide. Arrows indicate treatments application at sowing (A), urea application at side-dressing (U), and harvest (H).

At 90 days, there was a reduction in the cumulative net N_2O emissions of the DSi + DCD and urea-N treatments (Figure 2b). The same occurred at 150 days in DSs. This reduction in cumulative N_2O emissions, which was also observed in other studies, such as that of Bell et al. (2015), occurred at times of low N_2O emission (Figure 1a), in which fluxes were higher in the control treatment compared to the other treatments, resulting in a decrease in cumulative net N_2O emissions.

To facilitate the comparison of treatment effect on each crop, the cumulative annual N_2O emissions of all treatments were divided in periods: wheat, corn, and the post-harvest periods of both crops (Tables 2 and 3). The cumulative N_2O emissions in the wheat control treatment exceeded those from corn approximately three-fold (Table 3), despite the higher temperatures during corn cultivation (Figure 1d). This result was not expected and can be attributed to the fact that the area had been left fallow prior to wheat planting, growing weeds of several botanical species. It is likely that the killing of plants by herbicide application nearly 30 days prior to the installation of the experiment increased the availability of substrate to the denitrifying bacteria through shoot and roots. This result suggests that the initiating a no-tillage system in a fallow area may be an important source of N_2O to the atmosphere, even without the application of N fertilizer, as observed by Velthof and Mosquera (2011) and Aita et al. (2015).

In the average of all treatments, the winter crop contributed with approximately 52 % of the annual N_2O emission, while the contribution of the summer crop was of 28 %. Even if the N_2O emission observed in the control treatment was deducted from each treatment, the cumulative emissions of N_2O from the wheat crop still corresponded to 57 % of the total emission, compared to 43 % from corn. Despite the more favorable temperatures for microbial activity in corn than in wheat (Figure 1d), it is likely that this result is due to the lower occurrence of rainfall in corn in the first weeks after the application of N sources. This is the period of greatest N_2O production potential, because there was mineral N available in the soil and its consumption by wheat and corn was still small.

The annual average cumulative amounts of N_2O-N during the two periods after the harvest of wheat and corn accounted for 20 % of the total emissions, with no significant differences between treatments (Table 3). The low N_2O emissions, despite the rainy period between the harvest of wheat and corn planting, indicate that the denitrification process in the post-harvest period was limited by the low availability of substrates. As cultivation of two annual crops is restricted to tropical and subtropical climates, N_2O emissions in the post-harvest periods are still poorly understood and need to be better evaluated for different soil types and differences in the history of cultivation and fertilization of the areas.

Effect of the slurry application method

In the first 42 days of wheat cultivation, there was no significant difference in cumulative N_2O emission between the shallow-injected and surface-broadcast applications of DS (Table 2), contrary to results of studies in which higher emissions were found through injected pig slurry (Velthof and Mosquera, 2011; Aita et al., 2014) and cattle slurry (Ellis et al., 1998; Velthof and Mosquera, 2011). In this study, the lack of difference between the two methods of DS application in the winter was most likely related to the small amount of available N applied to the soil with DS (Table 1), which resulted in N_2O fluxes lower than 420 µg m^{-2} h^{-1} N (Figure 1a). As the WFPS values were higher than 80 % during this period and thus favorable for N_2O production (Linn and Doran, 1984), it is likely that the low temperature (Figure 1d) reduced the activity of the both denitrifying bacteria and heterotrophic microbial population responsible for the mineralization of organic N applied with DS (69.6 kg ha^{-1} N; Table 1). Thus, wheat probably assimilated the mineral N produced in this process and thereby limited the availability of substrate for denitrification. With surface-broadcasting (DSs), the contribution of DS in 42 days (Table 2) was equivalent to 37 % of the total emission (0-134 days; Table 3), and even lower with DSi (29 % of the total).
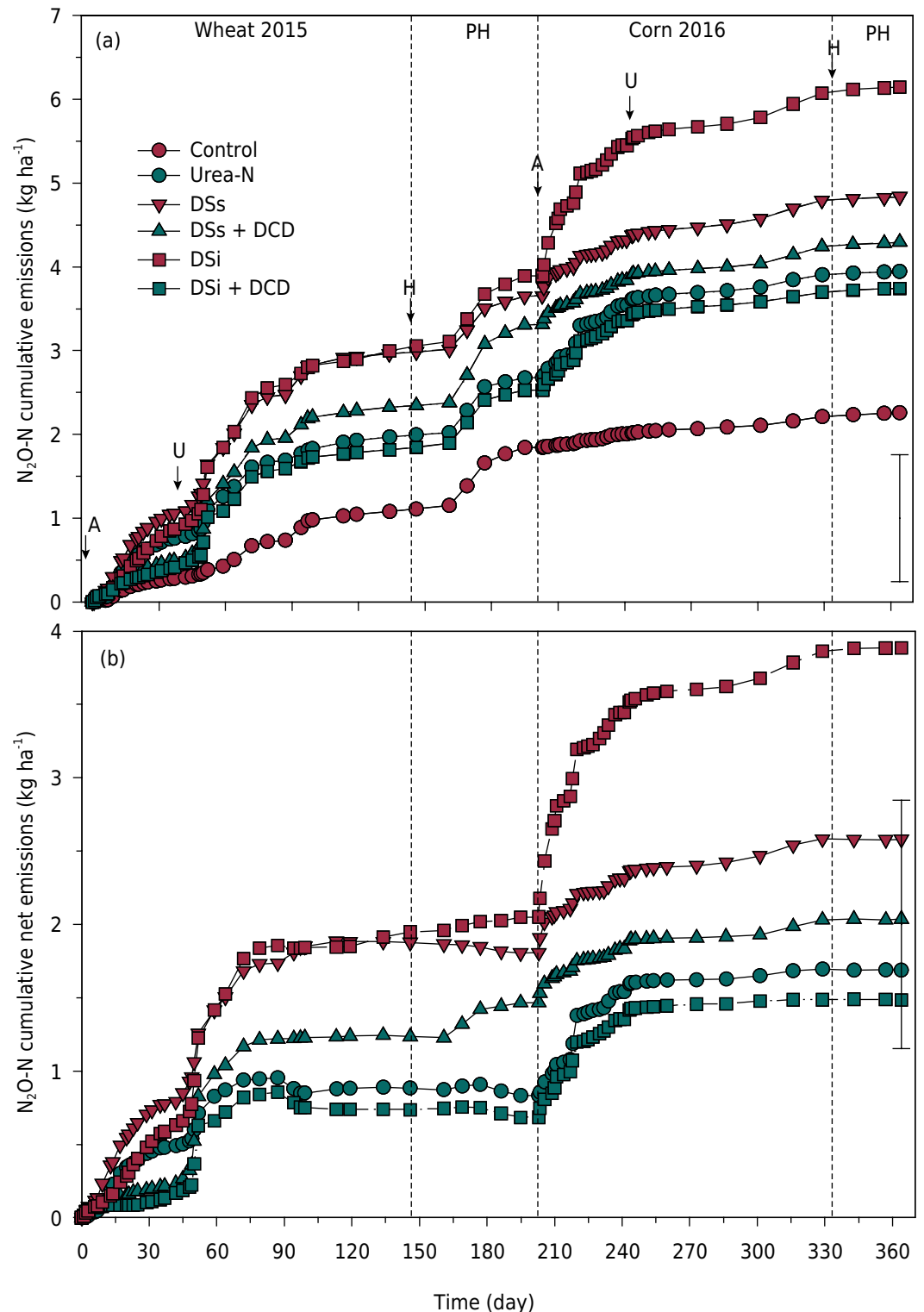
Figure 2. Cumulative emissions (a) and cumulative net emissions (b) of N₂O evaluated during the wheat and corn growing seasons and post-harvest (PH) periods. Urea-N = surface-broadcast urea-N (traditional N fertilization); DSs = surface-broadcast DS; DSi = Shallow-injected DS; DCD = dicyandiamide. Arrows indicate treatments application at sowing (A), urea application at side-dressing (U), and harvest (H). The vertical bars indicate the minimum significant difference between the treatments by LSD test at 5 %.

In corn, N₂O emissions in the first 34 days after surface-broadcasting and injection of DS were equivalent to 46 and 61 % of the total N₂O emissions occurring throughout the crop period, respectively. In the summer crop, DSi increased the cumulative emissions of N₂O in the first 34 days 2.5-fold (800 g ha⁻¹ N₂O-N), compared to DSs, in agreement with other studies with animal slurries (Velthof and Mosquera, 2011; Aita et al., 2014).

Table 2. Emissions of N$_2$O during wheat and corn growing seasons and post-harvest (PH)

Treatments	Wheat			PH Wheat	Corn			PH Corn
	0-42 days[1]	43-72 days[2]	73-134 days[3]	135-195 days	196-230 days[1]	231-260 days[2]	261-329 days[3]	330-364 days
	kg ha^{-1} N$_2$O-N							
Control	0.28 c[4]	0.39 d	0.41 ab	0.76 a	0.10 d	0.10 c	0.16 c	0.05 b
Urea-N	0.76 ab	0.85 b c	0.36 b	0.70 a	0.69 b	0.31 ab	0.23 bc	0.04 b
DSs	1.05 a	1.24 ab	0.55 a	0.69 a	0.52 bc	0.27 b	0.34 ab	0.05 b
DSs+DCD	0.49 bc	1.34 ab	0.49 ab	0.98 a	0.41 c	0.24 b	0.28 abc	0.05 ab
DSi	0.87 a	1.57 a	0.56 a	0.90 a	1.32 a	0.43 a	0.43 a	0.07 a
DSi+DCD	0.41 bc	1.08 ab	0.33 b	0.71 a	0.67 bc	0.29 b	0.20 bc	0.05 b

[1] Period between application of treatments and application of urea-N at side-dressing. [2] First period after application of urea-N at side-dressing. [3] Second period after application of urea-N at side-dressing. [4] Means followed by the same letters in the columns are not significantly different by the LSD test at 5 %. Urea-N = surface-broadcast urea-N; DSs = surface-broadcast DS; DSi = shallow-injected DS; DCD = dicyandiamide.

Table 3. Cumulative N$_2$O emissions during wheat and corn growing seasons and post-harvest (PH), and N$_2$O emission factor (EF)

Treatments	Wheat (0-134 days)	Corn (196-329 days)	PH Wheat + PH Corn (135-195 days + 330-364 days)	Annual (0-364 days)	N$_2$O EF
	kg ha^{-1} N$_2$O-N				% of N applied
Control	1.08 d [1]	0.37 d	0.81 a	2.26 c	-
Urea-N	1.98 bcd	1.23 b	0.75 a	3.95 b	0.73 ab
DSs	2.84 ab	1.14 bc	0.73 a	4.72 ab	0.81 ab
DSs + DCD	2.33 abc	0.93 c	1.04 a	4.29 b	0.67 b
DSi	2.99 a	2.18 a	0.97 a	6.14 a	1.27 a
DSi + DCD	1.82 cd	1.17 bc	0.75 a	3.74 bc	0.49 b

[1] Means followed by the same letters in the columns are not significantly different by the LSD test at 5 %. Urea-N = surface-broadcast urea-N; DSs = surface-broadcast DS; DSi = ahallow-injected DS; DCD = dicyandiamide; - = not applied.

This increase in N$_2$O emission caused by the injection of DS into the soil is explained by the fact that the slurry is concentrated within the injection slots, which can create an anaerobic environment, rich in inorganic N and readily available C (Comfort et al., 1990; Aita et al., 2014), stimulating N$_2$O production through the microbial processes of nitrification and denitrification (Baggs et al., 2000). Moreover, the reduction of N losses by NH$_3$ volatilization when the slurry is injected into the soil (Aita et al., 2014) increases the availability of mineral N, which can increase N$_2$O production during nitrification and denitrification (Velthof and Mosquera, 2011). The higher amount of mineral N injected into the soil in corn than in wheat (Table 1) and more favorable temperatures for microbial activity in summer (Figure 1d) may explain the distinct behavior observed between the two methods of DS application in the soil.

Effect of nitrification inhibitor

Considering that the inhibitory effect of DCD on nitrification is typically less than 30 days (Subbarao et al., 2006), the effect of adding the inhibitor to DS on N$_2$O emissions was compared in the period prior to the application of side-dressed urea (42 days in wheat and 34 days in corn).

The addition of DCD to DS at the time of application reduced N$_2$O emission in both crops, and this reduction was significant in both methods of DS application in wheat (0-42 days), while in corn (196-230 days) the inhibitor was only effective when DS was injected into the soil (Table 2). In wheat, the reduction in N$_2$O emission with DCD was 560 g ha^{-1} N$_2$O-N (53 %) for surface-broadcasting (DSs + DCD) and 453 g ha^{-1} N$_2$O-N (52 %) for injection (DSi + DCD). In wheat, the cumulative N$_2$O emission associated with the injected DS plus

DCD reduced emissions to levels that did not differ from the control treatment (Table 2). On the other hand, in corn the reduction in N_2O emission provided by DCD when DS was injected into the soil was equivalent to 49 % (648 g ha^{-1} N_2O-N). The higher efficiency of DCD in winter than in summer was also observed by Aita et al. (2015). This can be explained by the less favorable condition for the loss of N by NH_3 volatilization in winter, which results in a higher availability of N in soil as a substrate for N_2O production during nitrification and denitrification (Di and Cameron, 2012). Additionally, lower temperatures in winter than in summer (Figure 1d) may have resulted in the lower biodegradation of DCD (McGeough et al., 2016), improving the efficiency of the inhibitor.

This favorable effect of DCD in reducing N_2O emissions occurs by temporarily inhibiting the oxidation of NH_3 to nitrite (NO_2^-) in the first nitrification step, also temporarily reducing the availability of NO_3^- to denitrifying bacteria (Dell et al., 2011). When the inhibitor is degraded or leached in the soil, losing its efficiency, denitrification becomes controlled by the availability of C, which decreases as the slurry is degraded by the microbial heterotrophic population. Therefore, these results indicate that the addition of DCD to DS, especially when injected into soil, is an efficient practice in mitigating N_2O emissions in both winter and summer no-till crops.

N_2O emission factor

To calculate the N_2O emission factors (EF), N_2O emissions during each crop were considered, in addition to emissions during both post-harvest periods (Table 3), totaling 364 days. Except for DSi, where the EF value was 1.27 %, the EFs in the other treatments were less than 1 %, ranging from 0.49 % in DSi + DCD to 0.81 % in DSs. These EF values both for the urea-exclusive treatment (0.73 %) and treatments with DS application at sowing and side-dressed urea are slightly lower than the 1.0 % EF value proposed by the IPCC (2006).

For the method of DS application at sowing and the addition of side-dressed urea, there was no significant difference in the EFs between the injected (DSi) or surface-broadcast DS (DSs). Moreover, these two treatments did not differ from the traditional method of N application in form of urea to wheat and corn (urea-N). In relation to the addition of the nitrification inhibitor, it only significantly reduced the EF (in 2.6 times) when DS was injected into the soil (DSi + DCD) (Table 3). With this strategy of adding DCD to DS at the time of DS injection into the soil and supplementing the N required by the crops by adding side-dressed urea, the EF was reduced by 1.5 times compared to the exclusive use of urea (urea-N), although this difference was not significant, most likely due to the high variability in N_2O emissions. This result stresses the importance of the use of DCD in reducing N_2O emissions when animal slurry is injected into the soil, which has also been reported in other studies under different soil and climate conditions (Vallejo et al., 2005; Dell et al., 2011; Aita et al., 2014).

CONCLUSIONS

When dairy slurry (DS) is applied to the soil surface, with and without the nitrification inhibitor dicyandiamide (DCD), or when DCD is added to DS prior to DS injection into the soil, the annual N_2O emissions do not differ from those resulting from N fertilization of wheat and corn exclusively with urea.

The injection of DS into soil under no-tillage at sowing of wheat and corn combined with the posterior addition of urea-N at side-dressing increases annual N_2O emissions in comparison to the traditional use of urea as exclusive N source for crops.

The addition of DCD to DS only reduces annual N_2O emissions when DS is injected into the soil.

The use of urea as an exclusive source of N to wheat and corn under no-tillage or the strategy of combining DS application at sowing with the complement of N via side-dressed urea results in N_2O emission factors close to the value of 1 % proposed by the IPCC (2006). When DCD is added to DS prior to injection into the soil, the N_2O emission factor is approximately 50 % lower than the value proposed by the IPCC.

REFERENCES

Aita C, Gonzatto R, Miola ECC, Santos DB, Rochette P, Angers DA, Chantigny MH, Pujol SB, Giacomini DA, Giacomini SJ. Injection of dicyandiamide-treated pig slurry reduced ammonia volatilization without enhancing soil nitrous oxide emissions from no-till corn in Southern Brazil. J Environ Qual. 2014;43:789-800. https://doi.org/10.2134/jeq2013.07.0301

Aita C, Schirmann J, Pujol SB, Giacomini SJ, Rochette P, Angers DA, Chantigny MH, Gonzatto R, Giacomini DA, Doneda A. Reducing nitrous oxide emissions from a maize-wheatsequence by decreasing soil nitrate concentration: effects of split application of pig slurry and dicyandiamide. Eur J Soil Sci. 2015;66:359-68. https://doi.org/10.1111/ejss.12181

Amado TJC, Bayer C, Conceição PC, Spagnollo E, Campos B-HC, Veiga M. Potential of carbon accumulation in no-till soils with intensive use and cover crops in Southern Brazil. J Environ Qual. 2006;35:1599-607. https://doi.org/10.2134/jeq2005.0233

Anuário da Pecuária Brasileira - Anualpec. Anuário da pecuária brasileira 2014. 21. ed. São Paulo: Instituto FNP; 2014.

Baggs EM, Rees RM, Smith KA, Vinten AJA. Nitrous oxide emission from soils after incorporating crop residues. Soil Use Manage. 2000;16:82-7. https://doi.org/10.1111/j.1475-2743.2000.tb00179.x

Balem TA, Machado RL. Bases para o sistema agroecológico de produção de leite no sul do brasil. Asociación Latinoamericana de Sociología Rural (ALASRU). Grupo de trabalho eixo 2: Produtores rurais, agroecologia sustentabilidade: agroecologia e estratégia de reprodução social. 2014. https://www.alasru.org/pdf/ALASRU914final.pdf

Barcellos LAR. Avaliação do potencial fertilizante do esterco líquido de bovinos [dissertação]. Santa Maria: Universidade Federal de Santa Maria; 1991.

Bell MJ, Rees RM, Cloy JM, Topp CFE, Bagnall A, Chadwick DR. Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: effects of soil and climatic conditions and a nitrification inhibitor. Sci Total Environ. 2015;508:343-53. https://doi.org/10.1016/j.scitotenv.2014.12.008

Burkart MR, James DE. Agricultural-nitrogen contributions to hypoxia in the Gulf of Mexico. J Environ Qual. 1999;28:850-9. https://doi.org/10.2134/jeq1999.00472425002800030016x

Carozzi M, Ferrara RM, Rana G, Acutis M. Evaluation of mitigation strategies to reduce ammonia losses from slurry fertilization on arable lands. Sci Total Environ. 2013;449:126-33. https://doi.org/10.1016/j.scitotenv.2012.12.082

Comfort SD, Kelling KA, Keeney DR, Converse JC. Nitrous oxide production from injected liquid dairy manure. Soil Sci Soc Am J. 1990;54:421-7. https://doi.org/10.2136/sssaj1990.03615995005400020021x

Comissão de Química e Fertilidade do Solo - CQFS-RS/SC. Manual de adubação e de calagem para os estados do Rio Grande do Sul e de Santa Catarina. 10. ed. Porto Alegre: Sociedade Brasileira de Ciência do Solo; 2004.

Cui M, Sun X, Hu C, Di HJ, Tan Q, Zhao C. Effective mitigation of nitrate leaching and nitrous oxide emissions in intensive vegetable production systems using a nitrification inhibitor, dicyandiamide. J Soils Sediments. 2011;11:722-30. https://doi.org/10.1007/s11368-011-0357-0

Dalal RC, Wang W, Robertson P, Parton WJ. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Aust J Soil Res. 2003;41:165-95. https://doi.org/10.1071/SR02064

Dell CJ, Meisinger JJ, Beegle DB. Subsurface application of manures slurries for conservation tillage and pasture soils and their impact on the nitrogen balance. J Environ Qual. 2011;40:352-61. https://doi.org/10.2134/jeq2010.0069

Di HJ, Cameron KC. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? Soil Use Manage. 2012;28:54-61. https://doi.org/10.1111/j.1475-2743.2011.00373.x

Ellis S, Yamulki S, Dixon E, Harrison R, Jarvis SC. Denitrification and N_2O emissions from a UK pasture soil following the early spring application of cattle slurry and mineral fertiliser. Plant Soil. 1998;202:15-25. https://doi.org/10.1023/A:1004332209345

Gonzatto R, Chantigny MH, Aita C, Giacomini SJ, Rochette P, Angers DA, Pujol SB, Zirbes E, De Bastiani GG, Ludke RC. Injection and nitrification inhibitor improve the recovery of pig slurry ammonium nitrogen in grain crops in Brazil. Agron J. 2016;108:978-88. https://doi.org/10.2134/agronj2015.0462

Halvorson AA, Del Grosso SJ. Nitrogen source and placement effects on soil nitrous oxide emissions from no-till corn. J Environ Qual. 2012;41:1349-60. https://doi.org/10.2134/jeq2012.0129

Häni C, Sintermann J, Kupper T, Jocher M, Neftel A. Ammonia emission after slurry application to grassland in Switzerland. Atmos Environ. 2016;125:92-9. https://doi.org/10.1016/j.atmosenv.2015.10.069

Intergovernmental Panel on Climate Change - IPCC. 2006 IPCC Guidelines for national greenhouse gas inventories. Hayama, Japan: Institute for Global Environmental Strategies, 2006.

Jokela WE, Bosworth SC, Rankin JJ. Sidedressed dairy manure effects on corn yield and residual soil nitrate. Soil Sci. 2014;179:37-41. https://doi.org/10.1097/SS.0000000000000040

Klein CAM, van Logtestijn RSP, van de Meer HG, Geurink JH. Nitrogen losses due to denitrification from cattle slurry injected into grassland soil with and without a nitrification inhibitor. Plant Soil. 1996;183:161-70. c

Linn DM, Doran JW. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. Soil Sci Soc Am J. 1984;48:1267-72. https://doi.org/10.2136/sssaj1984.03615995004800060013x

Louro A, Sawamoto T, Chadwick D, Pezzolla D, Bol R, Báez D, Cardenas L. Effect of slurry and ammonium nitrate application on greenhouse gas fluxes of a grassland soil under atypical South West England weather conditions. Agr Ecosyst Environ. 2013;181:1-11. https://doi.org/10.1016/j.agee.2013.09.005

Maguire RO, Peter J, Kleinman A, Dell CJ, Beegle DB, Brandt RC, McGrath JM, Ketterings QM. Manure application technology in reduced tillage and forage systems: a review. J Environ Qual. 2011;40:292-301. https://doi.org/10.2134/jeq2009.0228

Martínez-Lagos J, Salazar F, Alfaro M, Misselbrook T. Ammonia volatilization following dairy slurry application to a permanent grassland on a volcanic soil. Atmos Environ. 2013;80:226-31. https://doi.org/10.1016/j.atmosenv.2013.08.005

McGeough KL, Watson CJ, Müller C, Laughlin RJ, Chadwick DR. Evidence that the efficacy of the nitrification inhibitor dicyandiamide (DCD) is affected by soil properties in UK soils. Soil Biol Biochem. 2016;94:222-32. https://doi.org/10.1016/j.soilbio.2015.11.017

Morley N, Baggs EM. Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. Soil Biol Biochem. 2010;42:1864-71. https://doi.org/10.1016/j.soilbio.2010.07.008

Murphy J, Riley JP. A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta. 1962;27:31-6. https://doi.org/10.1016/S0003-2670(00)88444-5

Rochette P, Angers DA, Chantigny MH, MacDonald JD, Gasser M-O, Bertrand N. Reducing ammonia volatilization in a no-till soil by incorporating urea and pig slurry in shallow bands. Nutr Cycl Agroecosyst. 2009;84:71-80. https://doi.org/10.1007/s10705-008-9227-6

Rochette P, Bertrand N. Soil-surface gas emissions. In: Carter MR, Gregorich EG, editors. Soil sampling and methods of analysis. 2nd ed. Boca Raton: CRC Press; 2008. p. 851-61.

Salazar F, Martínez-Lagos J, Alfaro M, Misselbrook T. Ammonia emission from a permanent grassland on volcanic soil after the treatment with dairy slurry and urea. Atmos Environ. 2014;95:591-7. https://doi.org/10.1016/j.atmosenv.2014.06.057

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3. ed. rev. ampl. Rio de Janeiro: Embrapa Solos; 2013.

Soil Survey Staff. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. 2nd ed. Washington, DC: USA: United States Department of Agriculture, Natural Resources Conservation Service; 1999. (Agricultural Handbook, 436).

Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Medgley PM. Climate change 2013: the physical science basis. Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press; 2013.

Subbarao GV, Ito O, Sahrawat KL, Berry WL, Nakahara K, Ishikawa T, Watanabe T, Suenaga K, Rondon M, Rao IM. Scope and strategies for regulation of nitrification in agricultural systems - challenges and opportunities. Crit Rev Plant Sci. 2006;25:303-35. https://doi.org/10.1080/07352680600794232

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. Análise de solo, plantas e outros materiais. 2. ed. Porto Alegre: Universidade Federal do Rio Grande do Sul; 1995. (Boletim técnico, 5).

Thompson RB, Meisinger JJ. Management factors affecting ammonia volatilization from land-applied cattle slurry in the Mid-Atlantic USA. J Environ Qual. 2002;31:1329-38. https://doi.org/10.2134/jeq2002.1329

Vallejo A, García-Torres L, Díez JA, Arce A, López-Fernández S. Comparison of N losses (NO_3^-, N_2O, NO) from surface applied, injected or amended (DCD) pig slurry of an irrigated soil in a Mediterranean climate. Plant Soil. 2005;272:313-25. https://doi.org/10.1007/s11104-004-5754-3

van der Weerden TJ, Luo J, Di HJ, Podolyan A, Phillips RL, Saggar S, Klein CAM, Cox N, Ettema P, Rys G. Nitrous oxide emissions from urea fertiliser and effluent with and without inhibitors applied to pasture. Agr Ecosyst Environ. 2016;219:58-70. https://doi.org/10.1016/j.agee.2015.12.006

van Groenigen JW, Kasper GJ, Velthof GL, van den Pol-van Dasselaar A, Kuikman PJ. Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications. Plant Soil. 2004;263:101-11. https://doi.org/10.1023/B:PLSO.0000047729.43185.46

Velthof GL, Mosquera J. The impact of slurry application technique on nitrous oxide emission from agricultural soils. Agr Ecosyst Environ. 2011;140:298-308. https://doi.org/10.1016/j.agee.2010.12.017

Webb J, Pain B, Bittman S, Morgan J. The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response - a review. Agr Ecosyst Environ. 2010;137:39-46. https://doi.org/10.1016/j.agee.2010.01.001

Wrage N, Velthof GL, van Beusichem ML, Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol Biochem. 2001;33:1723-32. https://doi.org/10.1016/S0038-0717(01)00096-7